IN THE UNITED STATES PATENT AND TRADEMARK OFFICE 1653

In Re Application of : Emil Israel KATZ

Serial No. : 09/982,172

Filed : 19 October 2001

For : PEPTIDES REPRESENTATIVE OF POLYPEPTIDES OF INTEREST AND ANTIBODIES DIRECTED THEREAGAINST, AND METHODS, SYSTEMS AND KITS FOR GENERATING AND UTILIZING EACH

#3
Plunkett
1-28-02

Art Unit : 1653

Examiner :

Attorney Docket No.: 01/22283

CLAIM OF PRIORITY DATE AND PRIORITY DOCUMENT

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Sir,

Applicant hereby claims the priority date of Israel Patent Application No. 140881 filed 14 January 2001 and encloses herewith a certified copy of that Israel Patent Application to support the claim for its priority date.

Respectfully submitted,

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16 January 2002
Ramat Gan, Israel

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בקשה לפטנט
Application for Patent

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מספר: Number	140881
תאריך: Date	14-01-2001
הוקדם/נדחה Ante/Post-dated	

אני, (שם המבקש, מענו – ולגבי גוף מאוגד – מקום התאגדותו)
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בעל אמצאה מכח היותי הממציא ששמה הוא:
Owner, by virtue of of an invention, the title of which is:

(בעברית) (Hebrew): שיטה לזיהוי וכימות חלבונים בביופסיה או בדגימת רקמה אחרת וערכה לשימוש בה

(באנגלית) (English): A Method For Identification And Quantification Of Proteins In A Biopsy Or Other Tissue Sample And A Kit For Use Thereof

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* בקשת חלוקה - Application for Division	* בקשת פטנט מוסף Application for Patent of Addition	* דרישת דין קדימה Priority Claim		
* מבקשת פטנט from Application	* לבקשה / לפטנט To Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האיגוד Convention Country
No. מס'	No. מס'			
Dated מיום	Dated מיום			

* יפוי כח: כללי/מיוחד – רצוף בזה / עוד יוגש
P.O.A.: general / specific - attached / to be filed later -

Has been filed in case הוגש בענין

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היום 14 בחודש January שנת 2001
This 14 of January 2001

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A METHOD FOR IDENTIFICATION AND QUANTIFICATION OF PROTEINS IN A BIOPSY OR OTHER TISSUE SAMPLE AND A KIT FOR USE THEREOF

שיטה לזיהוי וכימות חלבונים בביופסיה או בדגימת רקמה אחרת וערכה לשימוש בה

FIELD OF THE INVENTION

The present invention relates to a method for identification and quantification of proteins in a biological tissue sample. Furthermore said invention relates to a kit for identification and quantification of proteins in a biological tissue sample. More specifically, the present invention provides an immunoassay method for characterization of various types of high-incidence cancers, such as breast cancer, prostate cancer or lung cancer.

BACKGROUND OF THE INVENTION

Early detection of malignancy, when present, can often determine the outcome of the disease. In present art, when a malignancy is suspected, blood tests will be done to detect signs that point to an abnormality, and often a biopsy will be performed. A trained pathologist will view sections of the biopsy, search for chromosomal abnormalities, and perform immunocytochemisty to confirm the diagnosis and shed light on the specifics of the disease. Different mutations result in different tumors which present different protein antigens; different species of tumor and different sub-types within a tumor species act differently and will respond positively to different therapies. It is therefore vital to evaluate the nature of the tumor in order to decide on the course of therapy. This is currently a laborious process, since in immunocytochemistry there is a rate-limit on the number of antibodies that can be tested at once.

There is a need for a system to rapidly evaluate protein antigens in a tissue sample, such as in a biopsy taken from a suspected cancer patient.

It is the object of the present invention to provide a method and a kit for reacting the multitude of antigens present in a biological tissue sample with a large number of antibodies at once. The present invention allows identification and quantification of tumor-specific antigens, which aids in the diagnosis and characterization of the type of cancer present and in determination of the course of therapy. The present invention may be applied in characterization of the most common cancers, i.e. breast cancer, lung cancer and prostrate cancer, as well as in evaluation of the level of other proteins of commercial value in medicine and scientific research.

SUMMARY OF THE INVENTION

The present invention provides a method for identifying and quantifying proteins in a biological tissue comprising the steps of:

a) solubilizing a biological tissue sample with mild detergent, and then digesting the biological tissue sample;

b) mixing said digest of biological tissue with a plurality of labeled antigens capable of competing with the digestion products of the tissue sample proteins, for binding sites on antibodies bound to a solid support, incubating the mixture with the solid support;

c) washing the solid support to remove any non-specifically bound proteins;

d) adding to the solid support a color-producing enzyme bound to at least one molecule than can recognize and bind the labeled antigens;

e) adding to the solid support a substrate for said color-producing enzyme;

f) measuring the intensity of color on each area of the solid support using a device that measures color intensity;

g) analyzing the results using a computer program capable of identifying the amount of each protein present by comparing the intensity of color at a given position on the solid support, to a set of standards, and capable of correlating a given position on the solid support with the specific antigen-antibody complex it contains; thus receiving a true reading as to the amount present of each specific protein that was tested in the tissue sample.

The present invention further relates to a kit useful for identifying and quantifying multiple proteins related to various types of cancer in a biological tissue. The kit is comprised of a solid support to which are bound antibodies or antibody fragments capable of recognizing unique antigenic areas of various cancer-related proteins. Labeled antigens can be added that will compete with a digested tissue sample being tested, for binding sites on the solid support. The kit is further comprised of a color-producing enzyme bound to at least one molecule that can recognize and bind the labeled antigens, and a substrate for said color-producing enzyme. Results are read using a device that measures color, and a computer program capable of analyzing the results.

Further in accordance with one preferred embodiment of the present invention, said kit additionally comprises 2-3 control antibodies bound to the solid support, which are capable of binding polypeptide sequences expressed consitutively in all cells.

Moreover in accordance with one preferred embodiment of the present invention, said kit additionally comprises a series of control antibodies bound to said solid support, capable of binding descending predetermined amounts of labeled control peptides.

Still further in accordance with one preferred embodiment of the present invention, said kit additionally comprises a series of control antibodies bound to said solid support, capable of binding a series of labeled control peptides present as a mixture with a series of descending predetermined amounts of unlabeled peptide.

Additionally in accordance with one preferred embodiment of the present invention, the solid support is selected from the following:
plastic multi-well dishes, paper, nylon, glass, nitrocellulose and cellulose acetate, protein binding membranes, porous gels and polymeric films.

The present invention also provides a method for producing a kit that identifies and quantifies proteins in a biological tissue. The method of production is comprised of the following steps:

a) selecting a protein antigen of interest and using a computer program to identify 1-3 amino acid sequences within said protein that are unique to said protein.
b) digesting said protein to purify the unique polypeptides which can be used as antigens, or synthesizing the unique polypeptides by any means.
c) creating antibodies or antibody fragments against the unique polypeptide sequences using any conventional means.
d) fixing a plurality of said antibodies to a solid support so that each solid support can bind polypeptides unique to a multitude of proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

Figure 1 illustrates the first three steps taken to use a kit that identifies and quantifies the level of certain breast cancer-related proteins.

Figure 2 illustrates additional steps necessary to use a kit to identify and quantify the levels of breast cancer-related proteins.

Figure 3 illustrates the final steps necessary to use a kit to identify and quantify the levels of breast cancer-related proteins.

Figure 4 illustrates a graph depicting a calibration curve obtained by binding a known peptide, and the results of protein digests of two different cell lines, to an MDR1-specific proteomatrix.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

It is appreciated that the detailed description that follows is intended only to illustrate certain preferred embodiments of the present invention. It is in no way intended to limit the scope of the invention, as set out in the claims.

The present invention is based on the fact that many of the polypeptides released from any protein by proteolytic digestion are likely to be unique. Hence a specific antibody can be prepared that will recognize these unique polypeptides, and thus identify the protein. The chances of a unique polypeptide not being found in any particular protein are close to zero. There are 20 different possibilities at each amino acid position in the polypeptide chain. Given a sequence of 15 amino acids, the number of possibilities is 3.19×10^{19}, while there are only 10^7 different polypeptides of that length in the human genome.

The present invention additionally quantifies the level of expression of specific proteins in a tissue sample.

In the present invention, a set of proteins are chosen that are of interest, and the amino-acid sequence is scanned by computer program to identify up to three peptide sequences which are unique to said protein. Antibodies are raised in a laboratory animal against the unique polypeptide sequences, or antibodies or antibody fragments against the unique polypeptide sequences are created using any

conventional means. The antibodies are bound to a solid-support matrix, for example, a plastic multi-well dish, and the resultant device is known as a "proteomatrix".

To use the proteomatrix to identify and quantify protein levels in a given tissue sample, the polypeptides in a digested tissue sample are mixed with predetermined quantities of labeled commercial polypeptides. The mixture is added to the proteomatrix, and so the unknown polypeptides originating from the tissue sample being tested, compete with the labeled commercial polypeptides for binding sites on the proteomatrix. Since only the commercial preparation of polypeptides is labeled, the color intensity at a given area on the proteomatrix is an inverse measure of the amount of protein in question that is present in the tissue sample.

Referring to Fig. 1, a diagram is shown illustrating step-by step usage of a kit that identifies and quantifies the level of certain breast cancer-related proteins. In Step 1, a biopsy(1) is taken from a breast cancer patient, and 250 microliters volume of buffer, pH 8.5, is added. The mixture is heated for four minutes at 100°C. The proteolytic enzyme trypsin is added in an amount of 1 part trypsin to 20 parts (weight by weight) of the biopsy, and the mixture left to digest at 37°C overnight. This yields a terminal digest(2) of the proteins in the biopsy, broken down to its constituent peptides.

In Step 2, this digest is mixed with a sample of labeled peptides(3), represented in the figure by arrow-attached hexagons. The labeled peptides are specific for the proteins that the kit is designed to identify and quantitate (the "target" proteins). These peptides (the "chosen" peptides) have been chosen by scanning the database of the human genome to choose peptides that are unique tryptic fragments of the target proteins. All have been synthesized and then labeled at one end with biotin. In this figure, the circles, cylinders, non-arrowed hexagons and other shapes(4) represent the peptides in the biopsy digest.

In Step 3, this mixture is layered(7) onto the proteomatrix(5) which is a set of antibodies prepared against the chosen peptides that are derived from the target proteins, attached to an antibody-binding substrate, here a 96-well plastic dish. Each specific kind of antibody is attached to a separate well. In the inset to this figure can be seen the antibodies attached to the wells(6). Here two wells are depicted, each having a particular antibody attached to it. To certain wells are added antibodies

prepared against proteins (the 'control" proteins) that are ubiquitously present in tissue samples. To certain of these wells is added different but standard amounts of a peptide chosen to be uniquely present on that particular control protein, to provide a calibration curve for the entire proteomatrix.

Referring to Fig. 2, the next steps for using the proteomatrix device are shown. In Step 4, the mixture of chosen peptides, labeled peptides and attached antibodies(8) is left to react for two hours(9) at room temperature. The final situation of labeled peptides or unlabelled peptides (that compete with the corresponding label) attached to many of the antibodies is shown in the inset(10).

In Step 5, the proteomatrix is washed free of unattached peptides with a solution containing serum albumen or milk(11) so as to block any proteins that have not been bound by peptides or labeled peptides.

In Step 6, a solution(12) of horseradish peroxidase enzyme attached to streptavidin is added to the wells. The wells are again washed to remove unattached enzyme.

Referring to Fig. 3, the last two steps for using the proteomatrix device are shown. In Step 7, the proteomatrix is incubated for two hours(13) with the substrates of the peroxidase to develop a color(14) which is proportional to the amount of biotin-labeled peptide that had been bound to the antibodies in step 4.

In Step 8, the intensity of the color reaction(15) is measured in a conventional 96-well plate reader and the data analyzed by a computer program(16) which reports the amount of chosen peptide that was present in each of the wells, and there competed with the biotin-labeled peptide present.

Example 1

Example 1 is a description of steps followed in order to build and use a proteomatrix to test for multidrug resistance.

There are fifty proteins that are suspected as being responsible for the development of multidrug resistance in tumors. They are :

Gene of Interest and its Unigene Databank Access number

1. **P-glycoprotein (P-gp)** Hs.21330
2. **Multidrug Resistance(-associated) Protein (MRP-1)** Hs.89433
3. **Mitoxantrone resistance protein (MXR)** Hs.194720
4. **UPAR** Hs.83170
5. **TPA** Hs.213
6. **EGF** Hs.2230
7. **VEGF** Hs.73793
8. **KDR** Hs.12337
9. **FLT1** Hs.235
10. **bFGF** Hs.56066
11. **FGFR1** Hs.748
12. **Il-8** Hs.624
13. **p21** Hs.74984
14. **MDM2** Hs.89636
15. **p53** Hs.1846
16. **IGF-1** Hs.85112
17. **TGF-α** Hs.2023
18. **TGF-β** Hs.1103
19. **TS** Hs.82962
20. **PDGF** Hs.37040
21. **PDEGF** Hs.73946
22. **IFITH1** Hs.146360
23. **Cyclin-D1** Hs.82932
24. **Thrombospondin-1** Hs.87409
25. **Topo -2α** Hs.3378
26. **Topo-2β** Hs.75248
27. **MMP1** Hs.83169
28. **MMP-2** Hs.80343
29. **MMP-9** Hs.151738
30. **TIE1** precursor Hs.78824
31. **Gamma Actin** Hs.77443
32. **Beta-2-Microglobulin Precursor** Hs.75415
33. **PGK1** Hs.78771
34. **GADD-45** Hs.9701
35. **E2F** Hs.2331
36. **Rb** Hs.75770
37. **Sigma Receptor** Hs.24447
38. **Bub-1 homologue** Hs.98658
39. **fos** Hs.25647
40. **jun B** Hs.89792
41. **c-jun** Hs.78465
42. **myc** Hs.79070
43. **p73** Hs.247753

44. **ax** Hs.159428
45. **Bcl-2** Hs.79241
46. **Bcl-X** Hs.154969
47. **p27** Hs.238990
48. **Telomerase** Hs.115256
49. **Her-2/Neu** Hs. 148067
50. **UPA** Hs. 77274

Step 1) The above-mentioned proteins were to be cut using the enzyme trypsin. The theoretical tryptic fragments for each protein on this list were mapped, using the known sequence of the protein and the fact that trypsin cuts a polypeptide chain on the carboxyl side of a lysine or an arginine residue.

Step 2) The theoretical tryptic fragments of each of the above-mentioned 50 proteins were compared to all other known human proteins, to test for the novelty of their sequence. This was accomplished using "Blast Analysis" software via the Internet site www.ncbi.nlm.nih.gov/BLAST, which compares the peptide sequences to known sequences in the NCBI database.

Out of the above-mentioned list, P-glycoprotein (protein # 1 on the list above, also known as MDR1) was selected. P-glycoprotein is often present in cancer cells in later stages of malignancy, and thus its expression level is of diagnostic importance. The following 21 tryptic peptide fragments were found to be unique to this protein, since they were not found in any other human protein recorded in the human genome database Unigene Databank:

MDR1 Unique Tryptic Fragments

1) LYMVVGTLAAIIHGAGLPLMMLVFGEMTDIFANAGNLEDLMSNITNR
2) SDINDTGFFMNLEEDMTR
3) YAYYYSGIGAGVLVAAYIQVSFWCLAAGR
4) IGMFFQSMATFFTGFIVGFTR
5) LTLVILAISPVLGLSAAVWAK
6) AITANISIGAAFLLIYASYALAFWYGTTLVLSGEYSIGQVLTVF FSVLIGAFSVGQASPSIEAFANAR
7) LYDPTEGMVSVDGQDIR
8) ILLLDEATSALDTESEAVVQVALDK
9) NADVIAGFDDGVIVEK
10) LVTMQTAGNEVELENAADESK
11) SEIDALEMSSNDSR
12) EALDESIPPVSFWR
13) LNLTEWPYFVVGVFCAIINGGLQPAFAIIFSK
14) QNSNLFSLLFLALGIISFITFFLQGFTFGK
15) LAVITQNIANLGTGIIISFIYGWQLTLLLLAIVPIIAIAGVVEMK

16) FEHMYAQSLQVPYR
17) AHIFGITFSFTQAMMYFSYAGCFR
18) LMSFEDVLLVFSAVVFGAMAVGQVSSFAPDYAK
19) TPLIDSYSTEGLMPNTLEGNVTFGEVVFNYPTR
20) EANIHAFIESLPNK
21) GIYFSMVSVQAGTK

Another protein, MXR, was selected from among the 50 proteins responsible for the development of multidrug resistance in tumors. For MXR (protein # 3 on the list above), the following tryptic peptide fragments were found to be unique to this protein, not found in any other human protein as recorded in the human genome data base:

MXR Unique Tryptic Fragments

1) MSSSNVEVFIPVSQGNTNGFPATVSNDLK
2) AFTEGAVLSFHNICYR
3) EILSNINGIMKPGLNAILGPTGGGK
4) DPSGLSGDVLINGAPRPANFK
5) CNSGYVVQDDVVMGTLTVR
6) ENLQFSAALR
7) LATTMTNHEK
8) TSIGMELITDPSILSLDEPTTGLDSSTANAVLLLLK
9) TIIFSIHQPR
11) LFDSLTLLASGR
12) LMFHGPAQEALGYFESAGYHCEAYNNPADFFLDIINGDSTAVALNR
13) LAEIYVNSSFYK
14) EISYTTSFCHQLR
15) NLLGNPQASIAQIIVTVVLGLVIGAIYFGLK
16) AGVLFFLTTNQCFSSVSAVELFVVEK
17) LFIHEYISGYYR
18) ADAFFVMMFTLMMVAYSASSMALAIAAGQSVVSVATLLMTIC FVFMMIFSGLLVNLTTIASWLSWLQYFSIPR
19) YGFTALQHNEFLGQNFCPGLNATGNNPCNYATCTGEEYLVK
20) QGIDLSPWGLWK
21) NHVALACMIVIFLTIAYLK

Step 3) Peptide fragments were selected from among the unique tryptic fragments of MDR1 and of MXR, which are likely to be particularly immunogenic, based on their amino acid composition. A polyclonal antibody was prepared in rabbit, against peptide #8 on the MXR list. Monoclonal antibodies were obtained against peptides #8 and #19 on the P-glycoprotein list.

To calibrate the proteomatrix, as to protein which is bound in a non-specific manner, polyclonal antibodies were further prepared in rabbits, against three proteins known to be expressed constitutively in human tissue.

Step 4) Each of these antibodies was attached to a solid support, comprised of a well in a plastic tissue culture dish made by Nunc (Immunosorb). The antibodies were left to bind overnight at 4°C at a neutral pH in phosphate-buffered saline. The plastic dish was washed in phosphate-buffered saline containing 1 % bovine serum albumen, and stored at 4°C under sodium azide. This constitutes the proteomatrix kit, containing a matrix of antibodies each of which is specifically directed against a peptide, unique to that protein from amongst all of the proteins coded for by the human genome.

Step 5) A tissue culture of cells which are multidrug resistant to chemotherapeutic drugs, is dissolved and the proteins in the tissue are denatured by suspending them in 1-2% sodium dodecyl sulfate (SDS) for one hour, then precipitating the protein by adding methanol/acetic acid (pH 4) and leaving them overnight at -20°C. The precipitate is resuspended in 0.05-0.1% SDS. Trypsin is then added to this solution and left in contact for two hours at 37°C, to complete digestion of the protein. Sufficient bovine trypsin inhibitor is added to cause cessation of the proteolysis. This represents the digested tissue.

Step 6) A portion of the digested tissue is added to the proteomatrix and the two are left in contact for an hour to complete the antibody-antigen reaction. The digested tissue is washed twice with phosphate-buffered saline containing 1 % bovine serum albumen.

A mixture of all the tryptic polypeptides against which the antibodies were raised, is labeled with fluorescein at the amino-terminal ends of each peptide. The tryptic polypeptides are chosen so that when the mixture is applied to a fresh proteomatrix as specified, a uniform fluorescent signal is obtained in all the wells or positions in the proteomatrix. This mixture of tryptic polypeptides will compete with the digested tissue sample, for binding sites on the proteomatrix.

When such a mixture is applied to a proteomatrix which has been previously treated with a digested tissue, fluorescent signals will not be found in certain sites of the proteomatrix, if the tissue digest contains the peptides from a protein which the proteomatrix is designed to detect. The intensity of the reduction in signal, as compared with the mean of all the other positions in the matrix gives a quantitative measure of the amount of the protein present in the tissue, after correcting for the

amount of tissue that has been applied to the proteomatrix, using the signals from the three constitutive proteins used to calibrate the device, as described above.

Example 2

Example 2 is similar to Example 1, however Example 1 was a competition assay, with the color intensity measured, being inverse to the level of specific protein it represents. Example 2 is a direct assay, with the color intensity correlating the level of protein proportionately. The fluorescent label is applied to the directly to the ends of the peptides in the digested tissue, and the reaction with fluorescein is terminated by adding sufficient glycine. This processed, digested tissue sample is now applied to the proteomatrix and left as in Example 1, to complete the antibody-antigen reaction, washed as above and the fluorescent signal read. In this example, the intensity of the signal itself, as compared with the mean of all the other positions in the matrix, gives a quantitative measure of the amount of the protein present in the tissue, after correcting for the amount of tissue that has been applied to the proteomatrix, using the signals from the three proteins used to calibrate the device, as described above.

Example 3

Example 3 illustrates use of an MDR1-specific proteomatrix, to read the expression level of P-glycoprotein in two different Chinese Hamster Ovarian cell lines. One cell line expresses P-glycoprotein, while the other cell line does not.

Samples of 1700μg (by weight of protein) of cell membranes from a P-glycoprotein containing cell line and from a similar cell line that does not contain P-glycoprotein (a "wild type" cell line) were taken, suspended in 1.12 % SDS, precipitated with methanol/acetic acid, left at -20°C overnight, re-suspended in 0.07% SDS and then digested over night with TPCK-treated trypsin. The separate digests were centrifuged at 14,000 rpm for 25 minutes, and then spin-filtered on a Vivaspin column, to remove undigested material. Then the digest was applied at an amount equal to 500μg original membrane protein, in each of three wells of a 96-well cell culture dish that had been pretreated with 250μg of C494, a commercially available monoclonal antibody against P-glycoprotein. To other wells, in duplicate, were added decreasing quantities, of serial dilutions of the peptide MPNTLEGNVTK (using the one-letter notation for the amino acids), where all but the N-terminal lysine residue appear as

the central section of a presumed tryptic digestion product of P-glycoprotein. The serial dilutions were begun from a maximum of 500ng of peptide.

To all the wells were added 10ng of this same peptide, but biotinylated at the N-terminus. The whole was left to react, washed with 1 % bovine serum albumin and then reacted with streptavidin bound to horse-radish peroxidase. A color reaction of this enzyme measured the amount of biotin that was present in each well.

The optical density of each well of the 96 well dish was read using an Elisa reader.

Referring to Figure 4, a graph is shown plotting the individual optical density readings against the concentration of competing peptide that was placed in each well. This is a calibration curve. The data are fitted by a hyperbolic descending, three parameter equation, the parameters of which are used as the calibration for calculation of the amount of peptide present in each well of the digest. The horizontal lines on the figure represent the optical density of the mean of the three samples of the digest from the P-glycoprotein-containing membranes (lower line) and from the wild type membranes (upper line).

From the upper line it is seen that the wild type membranes, as expected, are devoid of P-glycoprotein since the digest gives no diminution of optical density as compared with the control, that is, there is none of the probe peptide in the digest. In contrast, the digest from the membranes containing P-glycoprotein (Pgp), gives a very significant diminution of the signal. From the calibration curve it can be calculated that each 500 μg of original membrane protein yields 0.052μg of peptide. Since the peptide has a molecular weight of about 1,000 daltons, and P-glycoprotein has a molecular weight (protein content) of 14,460, we can conclude that each well represents the digest from 9.33μg of P-glycoprotein protein. The Pgp content of the membranes from the P-glycoprotein-containing cells is thus some 1.64 %. From the turnover number of this enzyme and from the enzyme activity of an aliquot of the membranes, we calculate that the membranes contain 5.6 % P-glycoprotein on a protein for protein basis. Our assay thus recovers 29 % of the P-glycoprotein that was originally present. Control experiments, in which known amounts of the peptide were added to membranes before the digestion step and the resultant yield assayed using the proteomatrix as in the example, showed that the recovery was expected to be 45%.

The close accord of these two values shows that the proteomatrix described in this example successfully measures the amount of test protein, in a direct fashion.

CLAIMS

1. A method for identifying and quantifying proteins in a biological tissue comprising the steps of:

   a) solubilizing a biological tissue sample with mild detergent, and then digesting the biological tissue sample;

   b) mixing said digest of biological tissue with a plurality of labeled antigens capable of competing with the digestion products of the tissue sample proteins, for binding sites on antibodies bound to a solid support, incubating the mixture with the solid support;

   c) washing the solid support to remove any non-specifically bound proteins;

   d) adding to the solid support a color-producing enzyme bound to at least one molecule than can recognize and bind the labeled antigens;

   e) adding to the solid support a substrate for said color-producing enzyme;

   f) measuring the intensity of color on each area of the solid support using a device that measures color intensity;

   g) analyzing the results using a computer program capable of identifying the amount of each protein present by comparing the intensity of color at a given position on the solid support, to a set of standards, and capable of correlating a given position on the solid support with the specific antigen-antibody complex it contains; thus receiving a true reading as to the amount present of each specific protein that was tested in the tissue sample.

2. A kit useful for identifying and quantifying multiple proteins related to various types of cancer in a biological tissue, comprising a solid support to which are bound antibodies or antibody fragments capable of recognizing unique antigenic areas of various cancer-related proteins; the kit is such that labeled antigens can be added that will compete with a digested tissue sample being tested, for binding sites on the solid support; said kit is further comprising a color-producing enzyme bound to at least one molecule that can recognize and bind the labeled antigens; a substrate for said color-producing enzyme, a device that measures color, and a computer program capable of analyzing the results.

3. A kit to identify and quantify multiple proteins related to various types of cancer in a biological tissue, according to claim 2, additionally comprising 2-3 control antibodies bound to said solid support, capable of binding polypeptide sequences expressed consitutively in all cells.

4. A kit to identify and quantify multiple proteins related to various types of cancer in a biological tissue, according to claim 2, additionally comprising a series of control antibodies bound to said solid support, capable of binding descending predetermined amounts of labeled control peptides.

5. A kit to identify and quantify multiple proteins related to various types of cancer in a biological tissue, according to claim 2, additionally comprising a series of control antibodies bound to said solid support, capable of binding a series of labeled control peptides present as a mixture with a series of descending predetermined amounts of unlabeled peptide.

6. A kit to identify and quantify proteins in a biological tissue according to claim 2, wherein the solid support is selected from the following:
plastic multi-well dishes, paper, nylon, glass, nitrocellulose and cellulose acetate, protein binding membranes, porous gels and polymeric films.

7. A method for producing a kit that identifies and quantifies proteins in a biological tissue, comprising the steps of:
   a) selecting a protein antigen of interest and using a computer program to identify 1-3 amino acid sequences within said protein that are unique to said protein;
   b) digesting said protein to purify the unique polypeptides which can be used as antigens, or synthesizing the unique polypeptides by any means;
   c) creating antibodies or antibody fragments against the unique polypeptide sequences using any conventional means;
   d) fixing a plurality of said antibodies to a solid support so that each solid support can bind polypeptides unique to a multitude of proteins.

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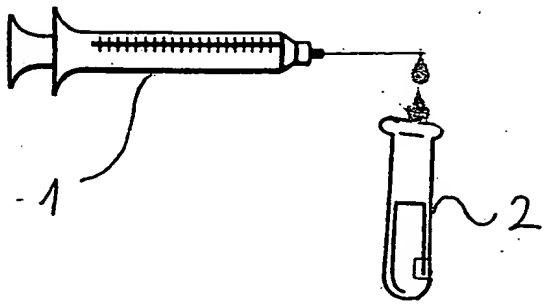
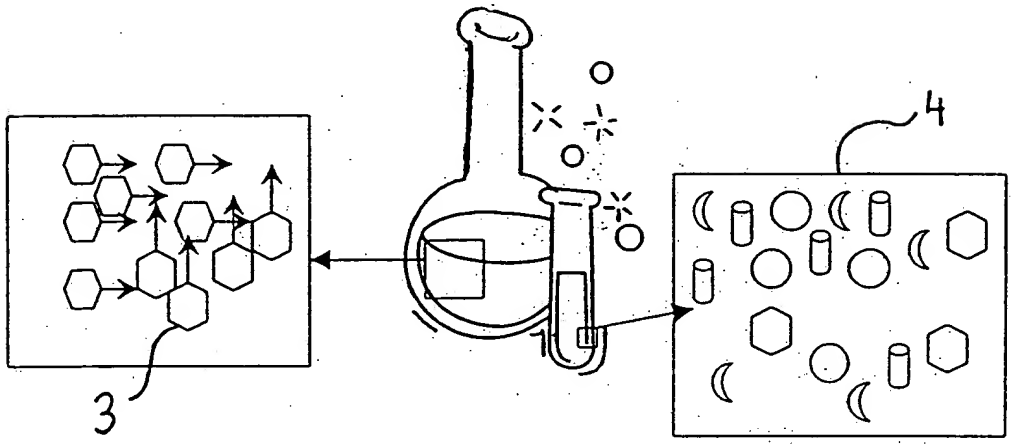
Step 1



Step 2



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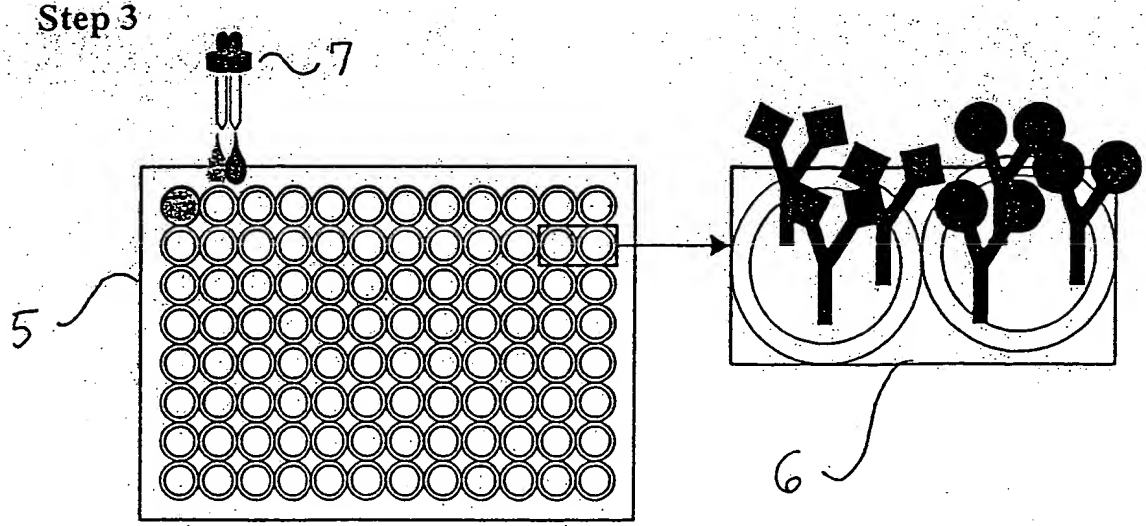
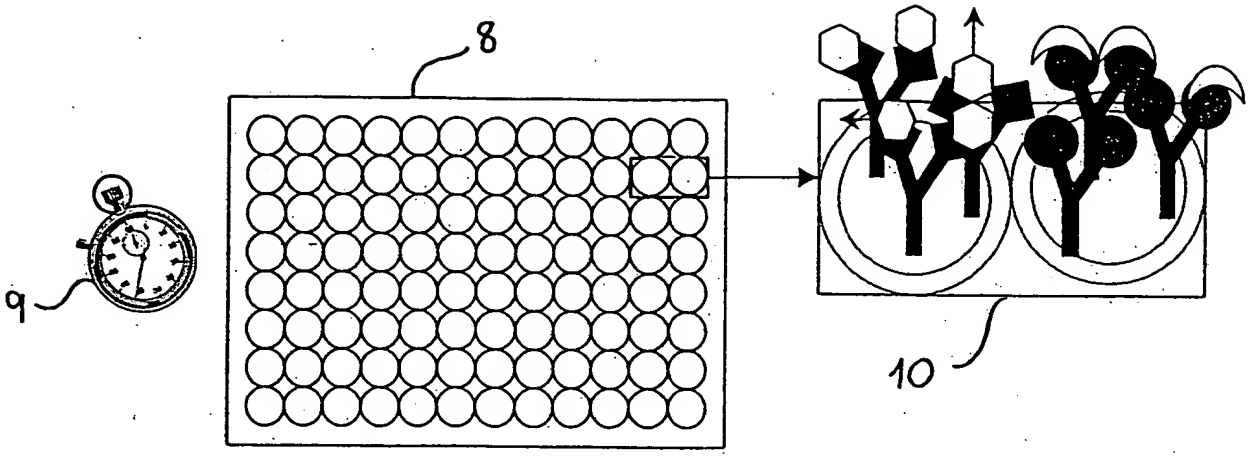


FIG. 1

Step 4



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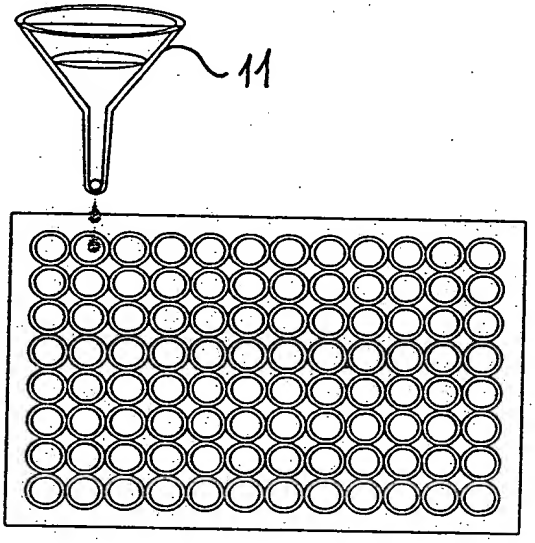

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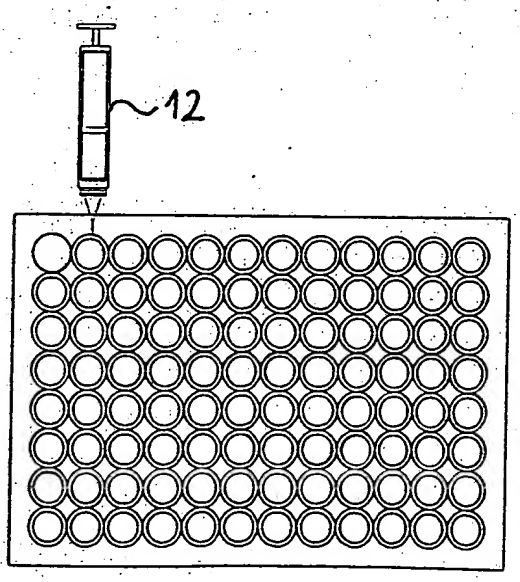
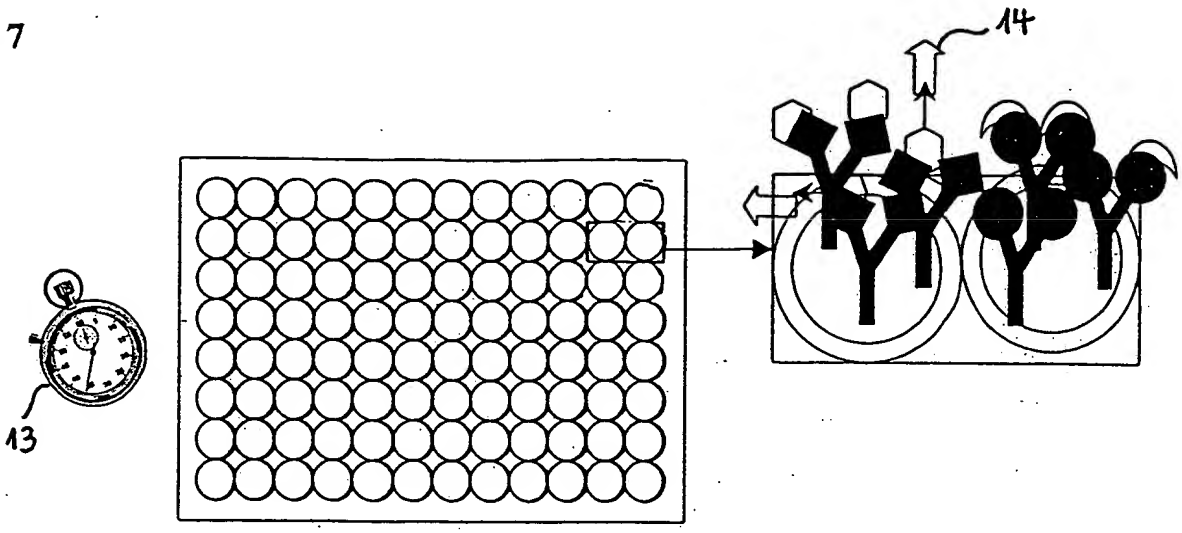


FIG. 2

Step 7



Step 8

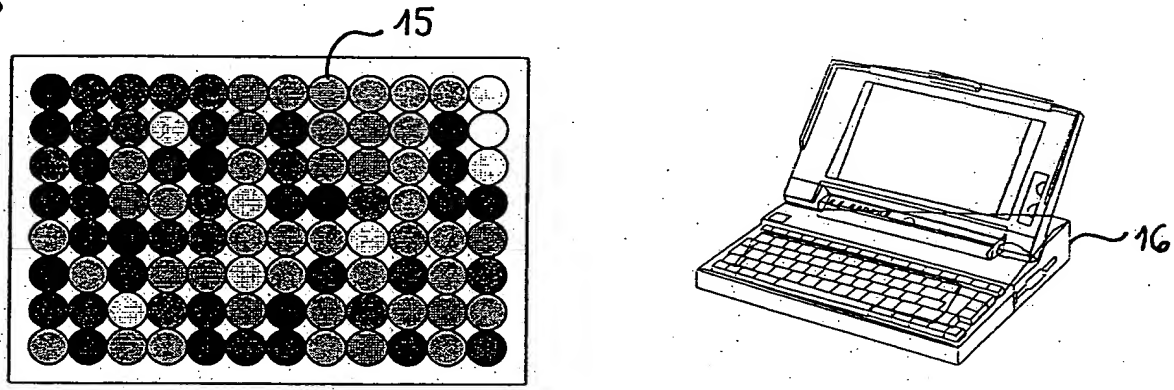


FIG.3

Binding Of Peptide Digests of Wild Type Or Pgp-Containing Cell Lines To An MDR1-Specific Proteomatrix

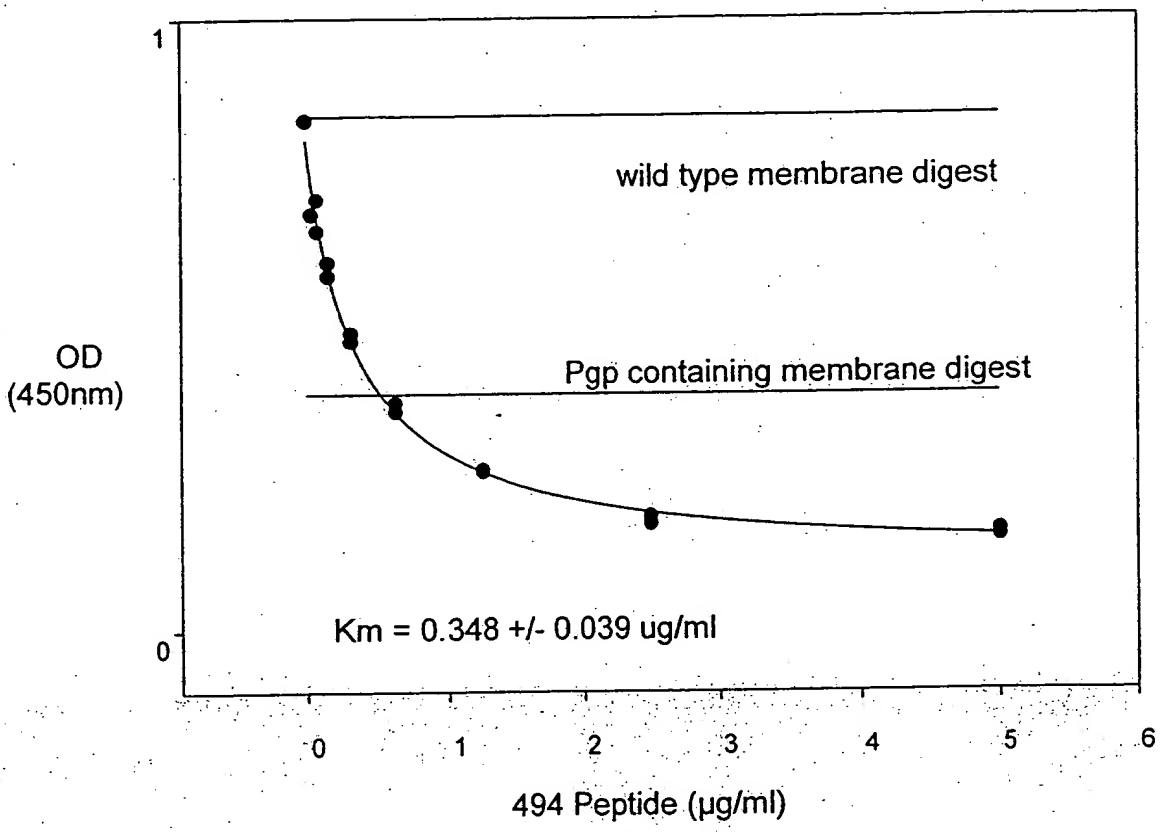


FIG.4